

NATURAL SOLUTIONS

Organic Answers to Toxic Questions

Carol Hunter

Just after midnight on March 24, 1989, the giant oil tanker *Exxon Valdez* ran aground on Bligh Reef off the coast of Alaska, spilling almost 21 million gallons of crude oil into the sea. The disaster caused an oil slick that covered 3,000 square miles and contaminated 1,090 miles of coastline along the pristine Prince William Sound, killing thousands of marine creatures and costing Exxon \$1.28 billion in clean-up costs.

While it was an environmental nightmare, the *Exxon Valdez* clean-up was also a large—and very public—field test of a technology known as bioremediation. When skimmers and other clean-up machinery could not handle the job, scientists at the Environmental Protection Agency added fertilizer to the beaches to stimulate the growth of indigenous microorganisms and speed the natural breakdown of the toxins, cutting the time to degrade the oil on Alaska's shoreline from five to 10 years to an estimated three to five years.

The high-profile clean-up efforts captured headlines in the *New York Times*, *Washington Post*, *Newsweek*, and other major media outlets. But bioremediation technology had been used long before *Exxon Valdez* ran aground for more insidious environmental disasters—gasoline leaking into groundwater from underground storage tanks or toxic chemicals from contaminated military sites seeping into lakes and streams.



The cost of cleaning up existing environmental contamination in the United States could be as much as \$1 trillion. (Photo: the Environmental Mining Council of British Columbia)

Scientists from many UC Berkeley departments and the Lawrence Berkeley National Laboratory have made significant contributions to the field of bioremediation. Three of these scientists, Professor John Coates, Dr. Hoi-Ying Holman, and Professor Norman Terry, demonstrate the wide range of research that can be put to task in the field, from new strains of bacteria and innovative monitoring techniques to genetically modified plants.

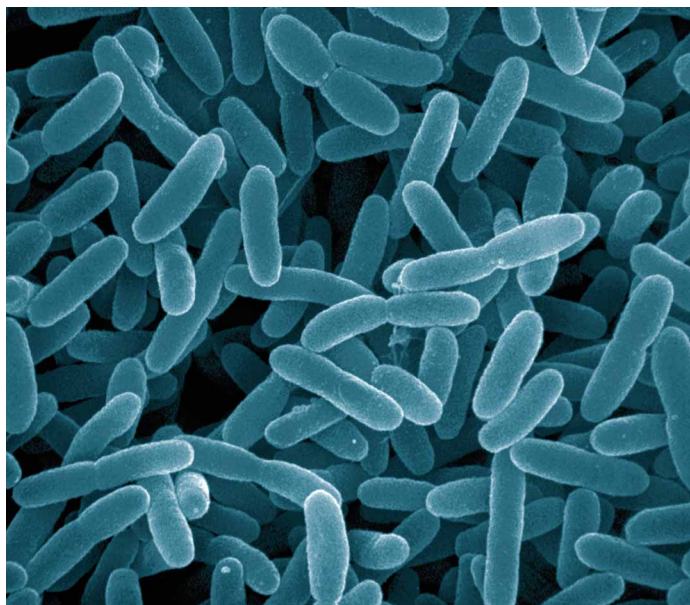
Putting Nature to work

Put simply, bioremediation is the use of biological organisms to return the environment to its natural state. Bioremediation in the form of decomposition has been around as long as life has existed, and human civilizations have been engineering systems such as compost piles and kitchen middens to take advantage of this natural process since at least 6,000 BCE.

These days, the term bioremediation usually describes more complex, scientifically engineered systems that use organisms such as bacteria, fungi, and plants to process toxic chemicals into less-toxic or non-toxic forms. Bioremediation has been used to clean up petroleum waste since the 1950s. Because petroleum is a naturally occurring, organic substance, the environment is full of microorganisms that can degrade it and often only require additional nutrients or an oxygen source to accelerate the process. More recent research has focused on other toxins: chemical solvents, heavy metals, and radioactive elements.

In 1980, the EPA started the Superfund Program to deal with these toxins by identifying and cleaning up sites that were so polluted they posed significant risk to the environment and public health. These sites are filled with contaminants such as vinyl chloride, chloroform, benzene, polychlorinated biphenyl (PCBs), mercury, lead, and others, many known to cause cancer, birth defects, and liver damage. The program has investigated almost 45,000 sites across the country and placed 1,500 sites on its National Priority List. The Bay Area is one of the toxic hotspots in the country, with 27 sites on the Superfund priority list, including the Hunter's Point Naval shipyard in San Francisco, the Alameda Naval Air Station, Lawrence Livermore Lab, and over a dozen semiconductor manufacturers in Santa Clara County.

The cost of removing these contaminants chemically or mechanically is prohibitive. The Superfund program alone has spent around \$40 billion to clean up just over half of the sites on the National Priority List, and, according to the US Geological Survey, cleaning up existing environmental contamination in the United States could cost as much as \$1 trillion. Many of toxins have leached away from their original source, contaminating rivers and lakes as well as aquifers



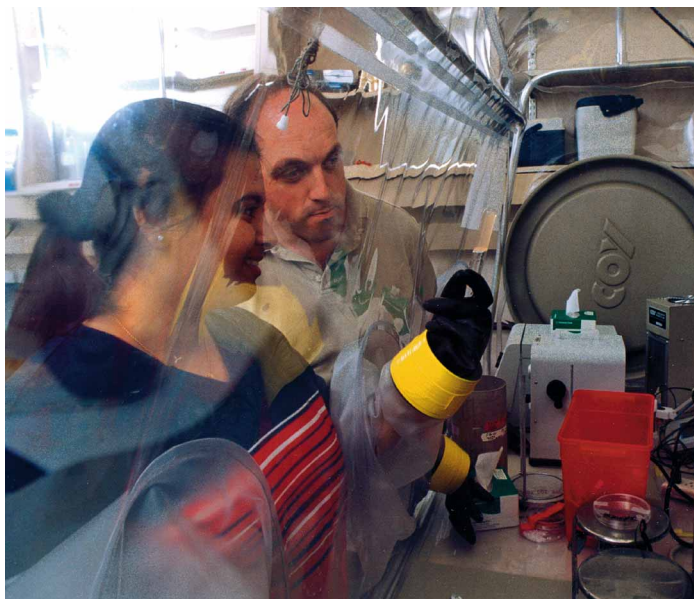
Dechloromonas strain RCB bacteria can break down two toxins, both perchlorate and benzene, in anaerobic environments. (Image: John Bozzola and Steven Schmitt, SIUC IMAGE Facility)

fers deep underground. A 1986 survey of groundwater found that 36% of more than 5,000 community water sources in the United States had organic contaminant concentrations above the maximum contaminant levels allowed for drinking water. Once a contaminant has infiltrated a large body of water, it is almost impossible to treat chemically. For these cases, bioremediation is the only affordable alternative.

Bacteria to the rescue

Dr. John Coates, a new professor in UC Berkeley's Department of Plant and Microbial Biology, has been researching the bioremediation of one of these groundwater contaminants, a chemical known as perchlorate. Used for solid rocket fuel as well as in explosives, bleaching agents, and defoliants, perchlorate interferes with iodine uptake into the thyroid gland and can cause fatal bone marrow disease and thyroid gland tumors. Because the thyroid plays an important role in development, perchlorate is especially dangerous for expectant mothers, since the fetus could suffer delayed development and learning disabilities.

Unlike petroleum contaminants, perchlorate is not naturally found in most environments. According to Coates, it's only known to occur naturally in the remote Atacama Desert in Chile. Scientists didn't expect many microorganisms to have evolved a way to break it down. "As of six years ago, there were only two known bacteria species that could process perchlorate," says Coates. "It was very poorly studied."



John Coates with graduate student Romy Chakraborty at Southern Illinois University. Chakraborty was the first person to isolate a bacterium that could break down benzene in oxygen-depleted environments. (Photo: J. Coates)

But once it was found in California drinking water in the late 1990s and recognized as a threat to public health, interest in the bioremediation of perchlorate increased dramatically. In 1997, Coates, then at Southern Illinois University, along with colleague Dr. Laurie Achenbach, began searching for more microbes that could break down the contaminant. Coates first developed a selective medium that would screen out everything but perchlorate-loving microbes. He then conducted a worldwide search for these bacteria in almost every type of environment, including contaminated and pristine soils, wetlands, aquifers, lake sediments, river sediments—even swine waste. “You name it, we probably looked there,” says Coates.

To their surprise, they found that these microbes were not rare, but were ubiquitous in the environment. “We found these bacteria in every site, even in Antarctica,” Coates says. He eventually isolated about 40 different species of the microorganism, all belonging to the phylum Proteobacteria. “The Proteobacteria is very broad, with five subdivisions,” says Coates. “This metabolic pathway was found in four of the five subdivisions. It is very phylogenetically diverse.” From an academic standpoint, these bacteria are intriguing due to their diversity and distribution in the environment. Some species can flourish in very harsh conditions, from acidic to basic, as well as high salinity. But their ability to break down perchlorate makes these organisms interesting from a practical standpoint as well.

Unlike many bacteria previously used for bioremediation, these proteobacteria eat away at the perchlorate in an anaerobic envi-

ronment. In fact, they *require* an anaerobic environment before they will start processing the chemical. Just as animals need to breathe air that contains oxygen to survive, many bacteria must absorb oxygen from their environment in order to complete their metabolic process and get energy from their food. But these proteobacteria will use perchlorate as an oxygen substitute when oxygen is not present.

In the field, engineers add a nutrient source like lactic acid to the contaminated area, stimulating the growth of all kinds of bacteria that quickly “breathe” all the available oxygen. Once the oxygen has been depleted, then the naturally occurring proteobacteria will start “breathing” perchlorate. But creating the right conditions to get things working can be tricky. If too little lactic acid is added, the bacterial growth will be limited by available food, oxygen will still be available, and the perchlorate-utilizing bacteria won’t process the perchlorate. But if too much is added, bacteria growth will be over-stimulated, meaning the microbial populations might munch through all the perchlorate and turn to other oxygen substitutes, altering the natural geochemistry of the environment. According to Coates, they would first use ferric iron, which can cause a bad taste in the water as well as the accumulation of rust in pipes, then they would turn sulfate to sulfide, creating a nasty “rotten egg” smell, and finally they would produce methane, a greenhouse gas.

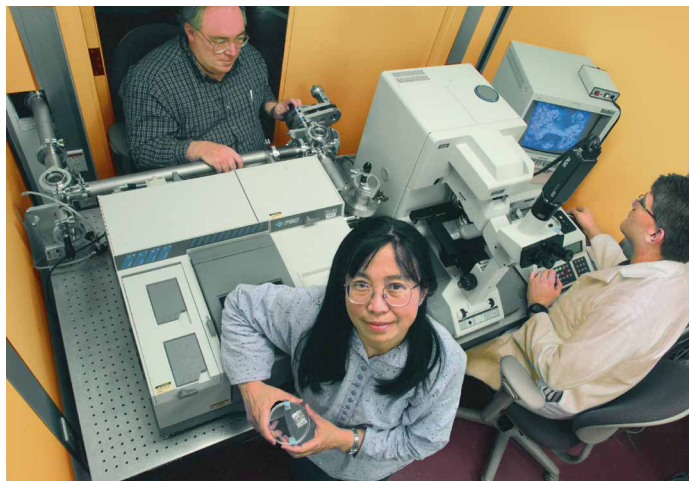
Once this delicate balance is achieved, however, the results are extremely positive. “For the field trials that have been done, perchlorate has been completely removed to all intents and purposes,” Coates says. “It was immeasurable or undetectable after stimulation in the field.” Coates is currently involved in two field trials, where his lab is following the remediative process more closely and carefully studying the microorganisms involved. Coates also discovered that these bacteria can remediate more than perchlorate. His lab has identified a particular strain of proteobacteria, *Dechloromonas* strain RCB, that can use the petroleum contaminant benzene as its food source at the same time it is using perchlorate as an oxygen substitute. Benzene is a dangerous, carcinogenic chemical often found deep in aquifers or soils where oxygen is not present. Not only do these particular *Dechloromonas* bacteria take care of two contaminants at the same time, they are also the first organisms discovered that can break down benzene in an anaerobic environment.

Spying on cells

One difficulty with bioremediation is that scientists don’t always understand what is going on in the field. As Coates puts it, there is a lot of “wait and pray” in the bioremediation business—you add bacteria or nutrients to an environment and then hope that the con-

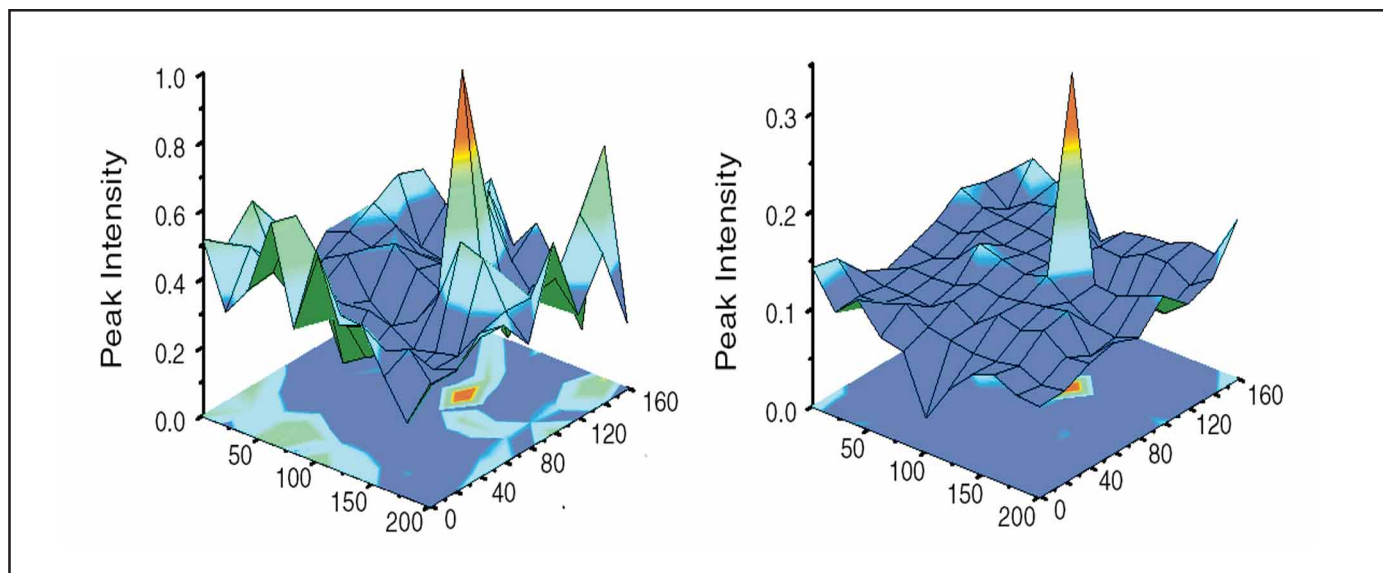
centration of toxins goes down. Because of the complex systems involved, it can be difficult to determine what exactly is causing a change. A team at Lawrence Berkeley National Laboratory has discovered a method that will take some of the guesswork out of bioremediation. Using a technique known as Infrared Spectromicroscopy, Hoi-Ying Holman and her colleagues Michael Martin and Wayne McKinney are able to watch the inner workings of cellular metabolism in real time.

On most days at LBL, you can find Holman hurrying up the 249 steps from her office to the experimental station inside the Advanced Light Source (ALS)—her daily workout routine, she jokes. She rushes past other stations with long thin metal tubes wrapped in tin foil transporting X-rays from the synchrotron in ultra-high vacuum, and finally arrives at her own small space. Specially designed optics capture the infrared beam—extremely bright but low photon energy—from the ALS, where it is bounced through a series of 20 mirrors and beamed through a combination optical/infrared microscope at the microscope stage incubator containing her experimental microbes. The bacteria inside, magnified about 300 times, appear on a computer monitor on the table nearby, along with masses of data showing the infrared measurements. Holman says she's eager every day to see what her microbes are up to. "We really get attached to our bacteria," she admits. "I always feel bad when we have to kill them."



Hoi-Ying Holman (center) with colleagues Wayne McKinney (left) and Michael Martin (right) at their experimental station inside LBL's Advanced Light Source. (Photo: LBNL)

But the amazing thing about Holman's technique is that she doesn't have to kill them at all. Most other methods of monitoring cellular mechanisms involve extracting the contents of cells, destroying them in the process, or using dyes or other agents that can affect the cell chemistry. Holman's infrared beam allows her and her colleagues to observe molecular reactions occurring inside cells in real-time, like a live movie. By analyzing the detailed characteristics of the infrared absorption bands produced by different compounds,



Unique spectral signatures show the spatial distribution of the biological protein amide II, indicative of the indigenous microorganisms (left), at the same location as reduced chromium, Cr(III) (right) after a four month period. (Image: LBNL)



Professor Norman Terry in the greenhouse. Terry is genetically modifying plants to remove selenium from the environment more efficiently. (Photo: Martin Sundberg)

Holman can tell exactly what chemicals are present and what reactions are taking place. Initial tests with human kidney cells demonstrated that the low energy of the infrared light does not affect cellular functions. The brightness of the ALS beam provides a spatial resolution of 2 to 12 microns, allowing the team to focus on a small colony of bacteria.

Holman started working on infrared spectromicroscopy in 1997. By early 1999, she had used her new method to follow the reduction of toxic metals among natural communities of bacteria taken from the Department of Energy's Idaho National Engineering and Environmental Laboratory's Radioactive Waste Management Complex. The team monitored and studied the biogeochemical transformation of hexavalent chromium, Cr(VI), into trivalent chromium, Cr(III), as it was occurring inside the living system. "We were the first on the planet to watch the bacteria in action as they detoxified chromium, like a play on stage," says Holman. "It was very exciting."

The bioremediation of a toxic metal like chromium is very different from the bioremediation of organic pollutants such as petroleum products or organic solvents. While these are complex organic molecules that can be broken down into harmless pieces, heavy

metals are atomic structures that can not be broken down further. Instead, bioremediation uses organisms to stabilize and immobilize heavy metals by changing their species—by adding or removing electrons to change the metal's valence state. Different species of heavy metals have different chemical, physical, and biological properties that have different impacts on public health.

Hexavalent chromium, a common industrial chemical used for chrome plating, dyes, leather tanning, and wood preserving, is found at two-thirds of the EPA's National Priority List sites. It is carcinogenic, mutagenic, highly soluble, and biologically active, easily crossing cell membranes and disrupting DNA replication inside cells. Trivalent chromium, on the other hand, is considered less dangerous because it is much less soluble and can not cross cell membranes.

At the Idaho waste site, high-level radioactive waste has been stored for more than 40 years, creating a toxic soup of inorganic metallic ions like hexavalent chromium, other inorganic ions, and radionuclides, as well as petroleum hydrocarbons and other volatile organic compounds. Over time, these toxins have seeped deep into the porous volcanic rock beneath the site. At the time of Holman's research, it was well known that the amounts of hexavalent chromium and other toxic metals were being reduced by natural processes at contaminated sites, but no one knew for certain whether this was due to a microbiological process or a geochemical reaction with the rocks.

To find out, Holman and her colleagues compared a sterilized sample rock under controlled conditions with another sample harboring a living community of *Arthrobacter oxydans*—bacteria that effectively reduce hexavalent chromium. Using the ALS beam, Holman found that, after five days, no reduction was taking place on the sterile rock sample and only small changes were measured in the sample with *A. oxydans*. But when the researchers added a weak solution of toluene, a petroleum chemical also found at the waste site, to the colonized sample, the infrared spectromicroscopy showed evidence of the reduction of hexavalent chromium, as well as degradation of the toluene where the bacteria were located.

To make sure the results accurately reflected what would occur in the field, Holman brought thin slices of basalt rock taken from 75 meters beneath the waste site, complete with their native microbial communities, into the lab. She exposed them to a hexavalent chromium solution and toluene vapor and watched them carefully under the ALS beam. After four months, spectromicroscopic graphic images showed colonies of bacteria at the same location as new trivalent chromium, where hexavalent chromium had vanished. The researchers also saw two new peaks that they believe were caused by

pentavalent chromium, Cr(V), an unstable, intermediate species of the metal. “Some of the intermediate compounds we saw can be more toxic,” Holman explains. “But in the past there was no way to see them because they are only stable in live systems.”

Holman has also used her new technique to examine the role microorganisms play in the detoxification of other carcinogens, the degradation of polyaromatic hydrocarbons, and the reduction of uranium. Her work has generated a great deal of interest in infrared spectromicroscopy, both nationally and internationally. Holman has even debunked some overstated bioremediation claims made on behalf of certain microorganisms. On one occasion she was given a sample of microbes that were supposed to be remediating contaminated soil, but didn’t seem to be having any effect. Holman put them under the infrared beam and confirmed that, indeed, they were not doing anything. “They were just sitting there,” she says. “They weren’t even dividing.” She later determined that the organisms were meant to work in aquatic environments and were inappropriate for their intended project, a discovery that saved a great deal of time and money.

Solutions from the greenhouse

Bacteria aren’t the only ones doing the dirty work. Phytoremediation—the use of plants to reduce or remove toxic chemicals from the environment—also holds great promise. Norman Terry, professor of environmental plant physiology at UC Berkeley’s Department of Plant and Microbial Biology, has focused his research on the phytoremediation of selenium for almost 15 years. He has discovered that using wetland plants such as cord grass, salt marsh bulrush, and rabbitfoot brush can be an effective, low-cost way to remove selenium from oil-refinery wastewater and agricultural drainage water. The key to his research is a mechanism in some plants and microorganisms that can turn selenium salts into the gas dimethyl selenide—a process known as volatilization.

Selenium is a naturally occurring metalloid that is abundant in the soils of California’s central valley. The detrimental effects of selenium made headlines in 1983, when scientists discovered that high concentrations of selenium at the Kesterson Reservoir in the San Joaquin Valley were causing dead and deformed waterfowl. But in low concentrations, selenium is harmless and in trace amounts it is actually a vital nutrient.

Terry started studying how volatilization occurs in plants in 1989 and found that, although the plants do not use selenium, the structure of selenium salt is very similar to that of

sulfate, which plants need to form vital sulfur-containing proteins. “The problem is that selenium compounds are chemically analogous to sulfur compounds,” said Terry, “and they tend to go through the same sulfate assimilation pathway in kind of a competitive way.” Because it mimics sulfur, selenium is also incorporated into sulfur-containing proteins, resulting in toxicity to plants and to the animals that ingest them. But not all of these elements are stored in the plant. Fortuitously, the same metabolic pathway that changes sulfur into dimethyl sulfide gas also enables plants to change selenium into dimethyl selenide gas.

This chance volatilization of selenium is of tremendous benefit when it comes to phytoremediation. Volatilization takes selenium out of the contaminated environment—the sediments, the water, and the biomatter—and puts it into the atmosphere. Eventually the selenium will come back down to earth, but since it is only harmful at high concentrations, this is generally not a problem. “Volatilization of selenium is an excellent way of cleaning it out of the system because it takes it completely out of a local area and puts it in another area where probably it’s going to be helpful rather than hurtful,” says Terry. “Toxicity spots are very, very localized. In the case of California, if it comes down somewhere else, chances are it will come down in an area that is suffering from selenium deficiency.”

Terry got his first field test of selenium volatilization in 1995 on an experimental wetland in Richmond run by the Chevron Corporation. The oil company had determined that their 35-hectare marsh



This experimental wetland in front of Chevron’s Richmond oil refinery can reduce the amount of selenium in the refinery wastewater by about three quarters. (Photo: Paul Kagawa/Chevron)

Feature

was successfully reducing the amount of selenium in its refinery wastewater by about three quarters, but the accumulation of concentrations in the sediments and plant biomatter could only account for about 70% of the missing selenium. They called in Terry to see if volatilization could account for the missing metalloid. Using small Plexiglas chambers on meter-square test plots in the wetland, Terry and his colleagues were able to capture dimethyl selenide being released from the plants and show that the plants and microbes were indeed volatilizing at a healthy rate. The research found that as much as 10–30% of the selenium could be volatilized from the marsh.

Terry thought that if wetlands could help remediate oil refinery water, they could probably help out with agricultural water in California's Central Valley as well, where heavy irrigation causes selenium to leach out of the soil and into the drainage water. After the Kesterson disaster, farmers were no longer allowed to flush water into drains and instead were forced to use huge evaporation ponds, which can be as large as 300 hectares. As the water evaporates, it leaves behind highly concentrated toxic salts.

In 1996, Terry's team joined the UC Salinity/Drainage Program—a joint research project involving scientists from UC Berkeley, Davis, and Riverside as well as the Tulare Lake Drainage District in Corcoran, CA. The researchers set up 10 quarter-acre wetland cells in the Central Valley and planted them with species such as cord grass, salt marsh bulrush, and rabbitfoot grass. Terry's research showed that the miniature wetlands removed an average of 69% of the selenium from the inflow, with most of that being stored in the sediments. But volatilization was also occurring, especially among certain plant species. The team found that in one summer month, the test wetland cell filled with rabbitfoot grass volatilized nearly half the selenium entering it.

Terry sees enormous potential in using engineered wetlands to reduce the selenium concentration in agricultural drainage water. But how would these experimental marshes avoid turning into another

environmental disaster like Kesterson? One major difference is that the filtering marsh would be a flow-through system instead of a closed system like the Kesterson reservoir. "In Kesterson, you were just pouring selenium in and giving it nowhere to go," Terry explains, "whereas with the flow-through systems you're trying to filter it out, but you've got a constant flow of water through there. That stops the selenium from building up to super-high levels." As part of a bioremediation project, the marsh would also be carefully monitored. Once the sediments and the plants became saturated with selenium, the marsh would have to be dried out, the selenium-filled plants mowed down, and the sediments either dug up and hauled away as toxic waste or remediated further with more selenium-tolerant plants.

Terry is trying to develop new super-selenium-loving plants using genetic engineering. "The problem is that plants at present work kind of slowly. It would take quite a few years for them to really significantly draw down the selenium pollution in the soil," he says. "What you want is to genetically engineer plants that will rapidly speed up this process, so

instead of taking 10 years or 50 years or 100 years to do it, you want them to do it in 2 or 3 years."

Terry has spent the last eight years genetically engineering plants such as Indian mustard (*Brassica juncea*) to tolerate, accumulate, and volatilize high levels of selenium, using genes from *E. coli* bacteria or other plants such as *Arabidopsis*. He is currently conducting the first field trials in the United States of plants genetically modified for phytoremediation, using genetically enhanced Indian mustard to remediate highly contaminated agricultural drainage sediments in a joint project with the US Department of Agriculture in Parlier.



Marsh plants like this rabbit foot grass (*Polypogon monspeliensis*) are very successful at removing selenium from the surrounding water. In one summer month during their research project in Corcoran, CA, Terry's team found that the test wetland cell filled with rabbit foot grass volatilized nearly half the selenium entering it. (Photo: Elkhorn Slough Foundation)

Terry has also been working with a plant called *Astragalus bisulcatus* or the two-grooved poison vetch—a small, slow-growing plant from the North American prairies that tolerates and accumulates extremely high levels of selenium. His team has identified one of the genes that give *Astragalus bisulcatus* its high tolerance for selenium and has transplanted this gene into *Arabidopsis* and the fast-growing, high biomass Indian mustard.

“What we will eventually do,” says Terry, “is to use the genes that we got from *Astragalus bisulcatus* and combine them with the genes that we’ve gotten from these other places and pop them into the same plant, trying to create kind of a super plant for phytoremediation. That’s the goal.” He hopes that, using genetic engineering, he will be able to create plants that can take up 10 to 100 times as much selenium as natural plants, greatly enhancing the chance for making plant-based remediation systems function quickly and economically.

The future of bioremediation

Over the last 10 years, significant scientific advances have been made in the field of bioremediation thanks in part to scientists like Coates, Holman, Terry, and others at UC Berkeley and Lawrence Berkeley Labs. Chemicals that were previously believed to be persistent in the environment can now be broken down or contained through proven bioremediation techniques. Bioremediation has moved far

beyond its initial application to petroleum contaminants to the remediation of organic and inorganic solvents, heavy metals, and radionuclides. Scientists hope that bioremediation will soon be successfully applied to the complex mix of toxics commonly found at waste disposal sites and Superfund National Priority List sites throughout the United States.

There are still many questions to be answered in the field of bioremediation—whether to use genetically modified organisms, when to add organisms to an area at all, and when regulators can simply sit back and monitor, letting nature take its course. But each year of research brings new discoveries and a better understanding of the hydrology and biogeochemistry of contaminated areas, of new species of microorganisms with unique metabolic pathways to break down toxins, and of the various environmental conditions such as oxygen level, pH, and available nutrients required to make these microorganisms function. Bioremediation is no silver bullet, but it is an important tool, offering a rapid, cost-effective, and environmentally friendly way to clean up contaminated environments. ■

Carol Hunter is a second-year graduate student at the UC Berkeley School of Journalism.

Want to know more?

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